

GENETIC EVIDENCE FOR DIPLOID MALES IN
HABROBRACON.¹

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The production of males from fertilized eggs in the parasitic wasp, *Habrobracon juglandis* (Ashmead), is discussed in a previous paper (Whiting, Anna R., 1925). It was shown that homozygous orange-eyed females, when crossed to related males with dominant black eyes, produced a few practically sterile black-eyed sons, in addition to the usual number of impaternal orange-eyed males and heterozygous black-eyed daughters. The few daughters of these "patroclinous" or biparental males were black-eyed, almost completely sterile and in many cases abnormal in appearance. It was postulated that a spermatozoön developing independently in the egg cytoplasm and crowding out the egg nucleus might produce a male which, although resembling its diploid sisters, would be haploid. This did not explain why recessive males failed to appear in broods from the reciprocal cross of homozygous black females by orange males; neither did it throw any light on the sterility of the biparental males and of their few daughters.

With the occurrence of new mutations additional facts have been brought out consistent with previous results and throwing further light upon the problem.

A series of quadruple allelomorphs affecting eye color (Whiting, Anna R. and Burton, Raymond H., 1926) has been used in various combinations in breeding experiments to be discussed. These are black (O), light (o^l), orange (o), and ivory (oⁱ), in decreasing order of dominance. In black, orange, and ivory the ocelli are of the same color as the compound eyes. In light, they are gray while the compound eyes remain black. Distinction from

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type is more easily made in females than in males and is especially clear in light females heterozygous for orange or ivory.

Three pairs of allelomorphs affecting the wings have also been used. Wrinkled (*w*), recessive to type (*W*), prevents complete expansion of the wings and often the normal development of legs and antennæ. It is almost completely recessive and somewhat lethal in the homozygous and azygous conditions. Reduced (*r*), completely recessive to type, reduces the wings, especially the primaries, in size and venation. No overlapping with type occurs. These mutations are discussed at length elsewhere (Whiting, P. W., 1926). Defective (*d*) (referred to in previous publication as d_{II95} , Whiting, P. W., 1924), like wrinkled, is almost completely recessive but shows some overlapping with type (*D*). It reduces the length or causes disappearance of the fourth branch of the radius vein (r_4) in 90–95 per cent. of pure stock under standard conditions. Unfortunately reduced and defective cannot always be recognized with certainty in wrinkled, while defective cannot be identified in reduced where the veins are so generally disarranged.

There is no linkage between any of these factors.

CROSSES PRODUCING BIPARENTAL MALES.

Table I. gives summaries of crosses which produce biparental males. Whenever significant differences involving locus *D* occur they are indicated in the formulæ. The impaternal haploid males arise from unfertilized eggs and resemble their mothers. Where the mothers are heterozygous these males fall into two classes as expected. Females show all the dominant traits of both parents as do the biparental males.

The first cross in the table will serve to illustrate this. Mothers are light, fathers black. All daughters are black, most sons light, the few biparental sons black.

In classes *c* and *e*, section I., mothers are defective (*d*), fathers normal (*D*). If numbers in these classes be combined it is found that 6 of the 58 biparental males are defective, 10.34 ± 2.70 per cent.; 753 of the 820 impaternal males, 91.83 ± 0.64 per cent.; 46 of the 569 females, 8.08 ± 0.77 per cent. Similarity of percentages in biparental males and females is apparent. The 13

light males produced in classes d and e give still further evidence of biparental inheritance. Their mothers were orange or ivory, fathers light. They resemble their heterozygous sisters as their ocelli are lighter in color than those of males from light stock.

TABLE I.
CROSSES PRODUCING BIPARENTAL MALES.

Section.	Parents.		Matings	Progeny.		
	♀.	♂.		Bipa- rental ♂♂.	Impa- ternate ♂♂.	♀ ♀.
I a	o ¹ o ¹	O	10	7	156	115
b	oo	O	246	489	11,221	8,436
c	o ¹ o ¹ dd	OD	32	55	764	544
d	oo	o ¹	8	10	276	326
e	o ¹ o ¹ dd	o ¹ D	1	3	56	25
f	o ¹ o ¹ i	o	11	32	744	545
II a	OOrr	OR	4	8	93	137
b	oorr	oR	4	5	47	54
c	o ¹ o ¹ rr	o ¹ R	9	9	45	92
III a	o ¹ o ¹ rr	o ¹ R	1	3	4	22
b	o ¹ o ¹ rr	oR	9	5	39	94
c	o ¹ o ¹ RR	Or	17	17	392	393
d	ooRR	Or	26	30	758	808
e	o ¹ o ¹ RR	Or	2	3	119	110
f	OOrr	oR	3	1	16	35
g	OOrr	o ¹ R	1	5	11	15
h	oorr	o ¹ R	6	8	82	78
IV a	ooddww	ODW	3	5	43	93
b	ooWW	Ow	9	19	643	440
c	ooddWW	ODw	14	29	660	404
d	o ¹ o ¹ ddWW	ODw	19	17	637	360
e	ooWw	OW	1	13	79	77
V a	ooWWrr	OwR	5	7	63	83
b	ooDDWWrr	OdwR	3	3	6	26
c	ooddWWrr	o ¹ DwR	3	4	17	70
d	oo ¹ WWrr	owR	3	3	41	48
Total			450	790	17,012	13,430

In section II. are data on crosses where parents differ in wing character, locus R, but are similar in eye color. Biparental males can be readily recognized by their non-reduced wings. These are of interest since they show that the chromosome carrying R acts in the same manner as those containing O and D.

In section III. are given summaries of crosses involving differences in O and R. Whether one or two recessive factors are present in the mother the biparental males show both dominant characters.

Section IV., deals with crosses involving differences in the O and W loci, and in some cases in D. Cross a, ooddww females by ODW males, gave 5 black-eyed males 1 of which was wrinkled. All had normal venation. Since homozygous wrinkled females have bred true in all cases tested by the author there is little doubt that the W is contributed by the father. All orange sons were wrinkled, practically all that could be identified were defective, and all black daughters normal as to wings.

Wrinkled sometimes occurs as a result of accident of growth. Since biparental males are abnormal in so many ways, as for example in their rather common habit of pupating without spinning a cocoon, their wings might have a greater chance to be wrinkled, thereby increasing percentage of wrinkled in this class.

Cross c, section IV., ooddWW female by ODw male, is of importance. A dominant factor affecting the wings is contributed by each parent. Of the 29 black non-defective sons, 25 were non-wrinkled while 4 had slightly wrinkled wings, a condition parallel with that found in the sisters except that percentage of wrinkled is higher. There was one wrinkled among the 660 orange males and 3 wrinkled among the 404 black females. Class d resembles this except that ivory females were used instead of orange. Results are similar. Nineteen matings gave 17 black males, 1 with wrinkled wings and 1 with defective venation, 637 ivory males, practically all defective, and 360 black females, 1 with wrinkled wings.

In section V. are types of crosses involving differences in O, W, R, and in two cases D. In class a, ooWWrr females by OwR males, there were produced 7 black, non-wrinkled, non-reduced males and 63 orange non-wrinkled reduced males. The females were like the biparental males except that 1 defective appeared. In class b the four differences are involved, two dominant factors contributed by each parent. The 3 biparental males were entirely dominant, the orange sons non-wrinkled and reduced, the daughters dominant except for two defective individuals. Wings of

biparental males show three dominants, two from the mother, one from the father.

Class c is likewise of special interest. Recessives d and r are contributed by the mother, o¹ and w by the father. Biparental males are orange, non-defective (with one exception slightly so), non-wrinkled, and non-reduced. Here again the wings of biparental males show three dominant characters, two from the father and one from the mother. In class d three females heterozygous for o and o¹, homozygous for W and r, are mated to owR males. All ivory males have reduced wings while among the orange males are the biparental males easily recognized by their non-reduced wings.

In all, four hundred and eighteen matings were made where female parent had recessive eye color, male dominant. 747 biparental males were produced in addition to 16,660 impaternal males and 12,901 females.

One hundred and thirty-five matings of defective females by non-defective males gave 307 biparental males, 6,325 impaternal males and 3,816 females. Defectives were recorded in one hundred and two of these matings. 30 defectives were found among 269 biparental males, 11.15 ± 1.29 per cent.; 4,744 among 5,159 impaternal males, 91.95 ± 0.25 per cent.; 175 among 3,085 females, 5.67 ± 2.81 per cent.

Three matings of ww females by W males gave 5 biparental males, 1 wrinkled, 43 impaternal males, all wrinkled, and 93 normal females.

Fifty-one matings of rr females by R males gave 61 biparental males, 462 impaternal males and 754 normal females.

TESTS OF BIPARENTAL MALES.

Table II. gives summary of tests of biparental males. Whenever possible they were tested by mating to homozygous recessive females. Individual males were often mated to several females. Since they produce but few daughters the results of a large amount of work seem meager.

Two hundred and forty-two males were tested. One hundred and ninety-seven or 81.40 ± 1.69 per cent. were found to be sterile. Of these, one hundred and thirty-nine were tested once,

forty-five twice, six three times, four four times, two five times, and one seven times, in all two hundred and eighty matings resulting in 23,089 sons, no daughters.

TABLE II.
TESTS OF BIPARENTAL MALES.

Probable Formule of ♂♂.	Source (See Table I).	Sterile ♂♂.	Sons of Mates.	Fertile ♂♂.	Daughters.	Sons of Mates.
Oo ¹	I <i>a</i>	4	396	0		
Oo.....	<i>b</i>	145	18,692	31	104	3,769
Oo ¹ Dd.....	<i>c</i>	4	145	0		
o ¹ o.....	<i>d</i>	2	56	1	5	38
o ¹ o ¹ Dd.....	<i>e</i>	1	72	0		
oo ¹	<i>f</i>	7	654	3	13	750
OORr.....	II <i>a</i>	1	159	1	21	66
ooRr.....	<i>b</i>	3	265	0		
o ¹ o ¹ Rr.....	<i>c</i>	7	346	1	1	25
oo ¹ Rr.....	III <i>b</i>	1	55	1	6	9
Oo ¹ Rr.....	<i>c</i>	5	137	2	6	160
Oo ¹ Rr.....	<i>g</i>	1	56	2	25	816
oo ¹ Rr.....	<i>h</i>	4	323	3	5	296
OoDdWw...	IV <i>a</i>	5	1,010	0		
OoDdWw...	<i>c</i>	4	705	0		
OoWW(w)...	<i>e</i>	3	18	0		
Total.....		197	23,089	45	186	5,929

Of the forty-five fertile males twenty-six were tested once each, seven twice each, four three times, two four times, three five times, one seven times, and two ten times, one hundred and two matings. Altogether these matings gave only 186 daughters among the 5,929 sons of the females, an average of 3.135 daughters for each fertile biparental male. Seven matings of one male made at two day intervals resulted in 21 daughters, 15 of these in one mating. Another male mated ten times at two-day intervals gave in five matings 21 daughters. These are the most prolific by a rather wide margin. Results indicate that more daughters could be obtained from biparental males by making repeated tests.

With two exceptions all biparental males produced daughters showing the dominant characters like themselves. They therefore breed like haplonts. This was true of the D, O, and R loci irrespective of the side from which the factors came. Although



nine biparental males carrying *w* were tested by crossing to *ww* females, some of them several times, no daughters were obtained among the 1,733 sons, all wrinkled like their mothers.

DAUGHTERS OF BIPARENTAL MALES.

Of the two exceptional biparental males mentioned above one occurred in a mating of ivory defective female by orange defective male. He had orange eyes and defective wings as would be expected. His mate was of the same genetic constitution as his mother. In the first culture bottle appeared 2 orange females, 1 with abnormal abdomen which died within the cocoon, the other sterile. In the fourth bottle was found an ivory defective female. She was fairly fertile. In the first culture bottle she laid over thirty eggs, one of which hatched into an ivory female. In the second bottle there was likewise a high mortality of eggs but 9 ivory males and 5 ivory females ultimately emerged. Her daughter from bottle a gave 49 males, another which lived but a short time gave 10 males. There are three possible explanations for the appearance of this ivory female. Her mother may not have been virgin when mated. This is improbable since great care was exercised in this matter and many more ivory females would be expected early in the life of the mother if she had mated with an ivory brother. The female may have been produced from an unfertilized egg, a phenomenon which has occurred but rarely in *Habrobracon*, or the male may have produced a spermatozoön not carrying the chromosome containing the *o* factor.

The comparatively late appearance of this female serves as an argument for the second explanation since daughters of biparental males usually appear in the first or second culture bottles. On the other hand the high mortality of her eggs might indicate that she is the daughter of the biparental male since the few females obtained from unfertilized eggs have proved highly fertile.

The second exceptional case was found early in the work by P. W. Whiting. It has not been previously discussed in detail. A male of type stock 1 was crossed with female of orange defective stock 3. 118 orange sons showed defect typical of stock 3. Black daughters were 15 normal, 1 defective; black sons were 3 normal, 1 defective, the defective among the black being due to

irregular dominance. One of these normal black males was mated to three orange females by each of which he produced a single daughter.

From one cross there were produced 30 orange males and in vial b a single black-eyed female of small size and with asymmetry of ventral abdominal sclerites. She produced only one larva which died. Whether she possessed D or d is not known. Her morphological abnormality and near sterility are comparable with such conditions in daughters of biparental males transmitting only dominant traits.

From another cross there were produced 92 orange males and in vial c a single orange female. She developed from a naked pupa and had asymmetrical sternites. She appeared normal in internal morphology and histology and produced 3 orange sons, 1 normal and 2 defective. Since her mother was of normal orange stock the occurrence of these indicates that she had received d from her father. Like many daughters of other biparental males she was of abnormal appearance, but unlike them she was somewhat fertile.

From the third cross there were produced 136 orange males and, in vial a, a single orange female. This female was normal in appearance and produced 71 offspring, males 13 normal, 16 defective, and females 23 normal and 19 defective. The defectives were due to the fact that this female's mother had d. Normal venation was isolated in later generations.

This biparental male had, therefore, in addition to black-bearing spermatozoa, two types of orange, od, the maternal combination, and oD, a recombination type. He is the only male found which breeds like a heterozygote. He and his daughters are not included in the following summary.

Of the 186 dominant daughters of biparental males only 121 were sufficiently normal to test. Most of these laid eggs which failed to hatch. Some lived for several days, stung the host caterpillars but laid no eggs. One gave a normal black male which proved sterile; one produced a black-eyed female pupa which died in the cocoon; one gave a morbid larva which died young and an abnormal female pupa with black eyes found dead in the cocoon; and another an abnormal pupa of uncertain eye-color and sex, and a fifth *five* larvæ which died and a female pupa of uncertain eye-

color. The dominant daughters have so far given only dominant offspring.

MORPHOLOGICAL ABNORMALITIES IN BIPARENTAL MALES AND THEIR DAUGHTERS.

Physical defects are rather common in biparental males and their daughters. These include abnormal sclerites in abdomen, defects in antennæ, abnormal legs, incomplete digestive tract, abnormal thorax, and genitalia. Among the 790 biparental males were found 41 or 5.19 ± 0.53 per cent. abnormal. Also there were 61 individuals that did not spin cocoons (called naked pupæ) 7.72 ± 0.64 per cent. This stands in contrast to conditions in impaternal males where there were but 24 abnormal and 83 naked pupæ among 17,111 normal, 0.14 ± 0.02 per cent. and 0.48 ± 0.04 per cent. respectively. Among the 13,430 sisters of biparental males there were 82 abnormal and 57 naked pupæ, 0.61 ± 0.04 and 0.42 ± 0.04 per cent. respectively.

In the daughters of biparental males abnormalities are often more extreme and present in a much higher percentage. 68 freaks and 23 naked pupæ appeared in addition to 95 normal. Percentage of freaks is 36.56 ± 2.38 , of naked pupæ 12.36 ± 1.63 . Brothers of these females showed 13 freaks and 2 naked pupæ to 5,914 normal, 0.22 ± 0.04 and 0.03 ± 0.02 per cents.

SUMMARY.

1. Four allelomorphs affecting eye color and three pairs of allelomorphs affecting wing form and venation, none linked, are studied from the point of view of the method of their inheritance by biparental males in *Habrobracon juglandis* (Ashmead).

2. A female homozygous for one or more recessive factors when crossed to a male carrying allelomorphs to these factors produces, in addition to recessive haploid sons and dominant diploid daughters, sons which have all dominant characters like their sisters.

3. In crosses where females are homozygous for some recessive and some dominant factors and males possess allelomorphs the biparental sons are entirely dominant, showing that they have some factors from each parent.

4. When three of these factors affect one structure (the wing in this case) if one recessive and two dominants are contributed by one parent, their allelomorphs by the other, this structure in biparental males shows all the dominant characters.

5. From these results it is concluded that biparental males are diploid (Whiting, P. W. and Whiting, Anna R., 1925) at least for the four chromosomes that can be identified genetically.

6. Biparental males and their daughters are often abnormal in appearance and usually sterile or nearly so. When fertile they breed as dominants (with one, and possibly two, exceptions noted above).

DISCUSSION.

When different types of crosses are made between inbred related stocks and results summarized it has been found that definite relationships exist between various percentages derived from these summaries.

Percentage of males among biparental offspring, (previously called percentage of patrocliny), $\frac{\text{biparental } \sigma\sigma \times 100}{\text{biparental } \sigma\sigma + \text{♀♀}}$, is negatively correlated with percentage of females, $\frac{\text{♀♀} \times 100}{\text{total}}$, and with percentage of total biparentals, $\frac{\text{biparental } \sigma\sigma + \text{♀♀} \times 100}{\text{total}}$. In

other words when a type of cross results in a high percentage of males among biparentals there is a low percentage of females and of total offspring from fertilized eggs. The female percentage is lower than it would be if the decrease were due only to the transformation of some fertilized eggs into biparental males. This indicates that there is a mortality of fertilized eggs in these crosses directly correlated with number of biparentals which are males. Types of crosses that produce no biparental males have the highest percentage of females.

The question as to why these individuals are males cannot be answered at this point. Dr. Castle has suggested that they may correspond to the intersexes which Goldschmidt gets in *Lymantria* (Goldschmidt, Richard, 1927). Some of the facts support this and it may be that the presence of certain genetic factors in some individuals causes sex reversal. No gradations have been ob-

served in *Habrobracon*. The biparental males have all been completely male in external and internal morphology and in reactions. The high mortality suggested above may be due to intergrades that cannot survive in this species. In *Lymantria* the intergrades occur as a result of wide crosses while in *Habrobracon* these males come from crosses of related stocks only.

Non-disjunction may also be considered a possibility. Biparental males may be diploid for all chromosomes save one, the sex chromosome, and thus be males although resembling their sisters in appearance.

Unfortunately *Habrobracon*, like the Hymenoptera in general, is not ideal for cytological study. Haploid number of chromosomes seems to be eleven and they are extremely minute. Nachtsheim has demonstrated (Nachtsheim, H., 1913) that in the honey bee chromosomes frequently fractionate so that somatic counts vary considerably being various multiples of the haploid number. The author finds indications of this in another form now under observation so that a very careful study and numerous counts are necessary before conclusions can be drawn.

In spermatogenesis of biparental males the first maturation division is abortive, the second apparently equational as in normal haploid males. This process may result in diploid spermatozoa which when united to recessive eggs would give only dominant triploid offspring. The high percentage of physical abnormalities in daughters of biparental males and their sterility may be due to their triploidy. They possess ovaries and ova normal in general appearance.

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